Plant Reproduction: Teaching a New Language of Love

Species-preferential proteins attract pollen tubes to female gametes in flowering plants. In a new study, *Arabidopsis* was taught to say “come hither” to maize pollen when it was engineered to express a maize attractant.

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The language of love is not universal. Humans dance or send text messages to get noticed. Birds and insects have species-specific songs that attract appropriate mates [1]. Organisms that spawn in open water like sea urchins express peptides that attract sperm of the right species, but are ignored by other species [2]. These diverse behavioral and molecular languages all function to bring opposite gamete types of the same species together for fertilization. Union of inappropriate gametes can lead to lethality or to the production of sterile hybrids. Consequently, the mechanisms that bring gametes together can be highly specific, even species-specific — they reinforce boundaries between species and prevent hybridization [3]. Plant breeders have a different agenda and would like to make the widest possible crosses to increase their access to useful genetic diversity. Can the molecular language of gamete attraction, once understood, be used to make novel hybrids? This is a difficult challenge and many barriers, before and after gamete fusion, would have to be overcome. A paper published in this issue of *Current Biology* [4] provides an elegant path over one of these barriers.

Flowering plants have immotile sperm that are carried to female gametes by a pollen tube (Figure 1). Two sperm develop within each pollen grain, which are deposited on the stigma by animals (e.g. bees, hummingbirds, plant breeders), when they contact the stigma directly, or when carried by wind. The stigma captures pollen and supports germination of the pollen tube, which extends through the style, enters the ovary, and targets an ovule (Figure 1). Two sperm are released in the ovule; one fuses with the egg to produce a zygote, the other with the central cell to form endosperm, a tissue that supports embryo and seedling development [5].

Molecules that attract pollen tubes to ovules have recently been identified and, so far, they are all small, cysteine-rich polypeptides. The attractant chemocyanin directs lily pollen tubes from the stigma into the style [6]. ZmEA1 (from maize [7]) and LURE proteins (from *Torenia* [8]) are expressed by specialized synergid cells that flank the egg (Figure 1B), and are essential for pollen tube attraction [9]. These proteins attract pollen tubes through a small opening in the ovule (micropyle) so the pollen tube can release sperm near female gametes. Flowering plant genomes encode large numbers of these small proteins [10], most with no known function, indicating the potential for complex combinations creating a specific language of attraction.

Some insights into the exclusivity of pollen tube attraction have already been gained. Purified *Torenia fournieri* LURE proteins attract *T. fournieri* pollen tubes *in vitro*, but not *Lindernia micrantha* (a closely related species) [8]. When *T. fournieri* pollen tubes were confronted with a pair of ovules *in vitro*, they consistently chose their own species’ ovule over that of a related species [11]. So, when pollen of a different species lands on a foreign stigma, it may be able to germinate and extend a tube into the flower, but if the language of attraction is not recognizable, the tube will not be able to locate an ovule and deliver sperm (Figure 1A).

In this new study, Mihaela L. Márton and colleagues, in Thomas Dresselhaus’ group at the University of Regensburg, taught *Arabidopsis* a single phrase from the maize language of attraction [4]. To achieve this, they expressed the maize pollen tube attractant as a GFP fusion protein (ZmEA1–GFP) from an *Arabidopsis* promoter that drives expression in synergid cells [12]. ZmEA1–GFP signal was only detectable in transgenic

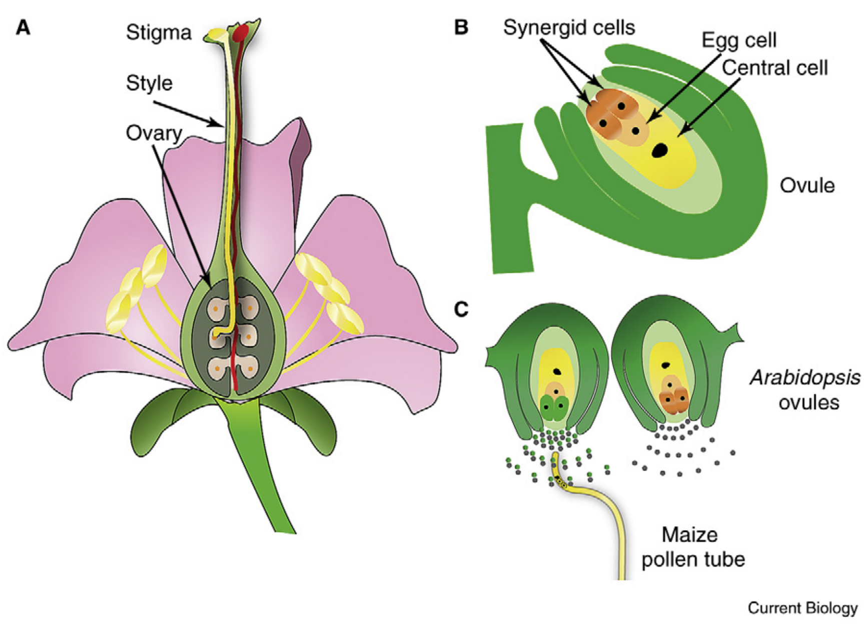

Figure 1. Pollen tube attraction is specific.

(A) A flower schematic with two pollen grains on the stigma. One is from the appropriate species and germinates a yellow pollen tube that is attracted to an ovule. The red pollen grain is from a different species and is unable to target ovules. Anthers (also yellow) are the site of pollen development. (B) This ovule schematic highlights critical reproductive cells. (C) Márton *et al.* [4] found that when an *Arabidopsis* ovule expresses maize pollen tube attractant (ZmEA1–GFP, gradient of green dots) it can attract a maize pollen tube. *Arabidopsis* ovules expressing their own attractants (grey dots) did not. The pollen tube contains two sperm cells and a nucleus (black). Illustration by Judith Nathanson, Brown University.

Arabidopsis when the synergid promoter was used; egg and central cell promoters failed. Synergids appear to have a specialized secretory system that has evolved to pump out pollen tube attractants [12].

Arabidopsis ovules expressing ZmEA1–GFP were placed next to wild-type *Arabidopsis* ovules in an *in vitro* system where maize pollen tubes could be grown (Figure 1C). In control experiments, one of the two *Arabidopsis* ovules expressed GFP, and only about 15% of maize pollen tubes headed toward it, and these kept growing past the ovule. In contrast, >50% of maize pollen tubes were attracted to an *Arabidopsis* ovule expressing ZmEA1–GFP; of these, ~30% stopped very near the micropyle.

This result shows that expression of a single molecule is sufficient to re-orient the direction of an extending maize pollen tube so that it would grow toward an *Arabidopsis* ovule. As representatives of the two classes of flowering plants, maize (monocot) and *Arabidopsis* (dicot) shared a common ancestor ~150 million years ago [13], suggesting that pollen tube guidance in all flowering plants may be governed by a system of attractants that can be transferred from one species to another.

Can pollen tube attraction be engineered for extreme plant breeding? Plant breeders introduce useful traits (e.g. disease resistance, drought tolerance, nutrition) to crop plants by cross-pollination followed by selection of recombinants with desired traits. Prospects for continued improvement of crops are diminished when the group of plants that can be crossed with each other is limited or lacks genetic diversity [14]. The experiments published by Márton *et al.* begin to test the concept that reproductive systems could be engineered so that genomes from two plants of different species, genera, perhaps even family, order, or class, could be combined.

Márton *et al.* have shown that pollen tubes of maize can be brought to an *Arabidopsis* ovule. This result suggests that barriers to extremely wide crosses can be overcome, but many significant challenges remain. Maize pollen tubes stopped at the *Arabidopsis* micropyle, but did not enter it, and did not burst to release sperm, so it was not possible to evaluate whether a zygote containing a maize/*Arabidopsis* genome could be produced. The signals that instruct the pollen tube to burst and release sperm are also specific [15], and there is evidence that molecules required for sperm to fuse with the egg and central cell are not compatible between divergent species [16]. Additional challenges will be to overcome embryo lethality caused by aberrant chromosome segregation and imbalance of gene dosage in hybrids. While these obstacles are significant, this is an exciting time for plant reproduction research, and our understanding of the critical mechanisms is increasing rapidly. With continued progress, the goal of engineering reproductive systems to produce novel plant genomes could be achievable.

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Genome Evolution: Where Do New Introns Come From?

A new study reports creation of spliceosomal introns in multiple related fungal species by proliferation of cryptic elements. Resonances to a case in unrelated algae suggest such elements hold general answers to long-standing mysteries of intron evolution.

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The discovery 35 years ago that the DNA encoding an mRNA can be interrupted by introns — intervening sequences that are removed from transcripts — was one of the most unexpected discoveries in the history of molecular genetics [1]. This discovery immediately raised a host of questions that remain debated to this day. Do introns have a general function? What are the fitness